1299-Pos Board B250
Decoding the Role of Receptor Dimerization in Plexin-Semaphorin Signaling
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Plexin-semaphorin signaling plays an important role in the formation of neural networks by acting as the guidance cue for axon growth or retraction. The plexin receptor is a single-pass transmembrane protein with a sema domain at the extracellular N-terminus and a GTP-ase activating protein domain on the cytoplasmic C-terminus. Binding of semaphorin is known to activate the plexin receptor, but the mechanism of how that binding event leads to activation is not understood. We will present our recent work using pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) to measure plexin protein-protein interactions in live cells. PIE-FCCS transforms fluctuations in fluorescence intensity (arising mainly from diffusion) into information about a protein's mobility and concentration. By cross-correlating the fluorescence fluctuations in two color channels, PIE-FCCS also quantifies co-localization and co-diffusion with high accuracy. This method has been used to resolve the mechanism of epidermal growth factor receptor (EGFR) activation and inhibition, as well as the dimerization constant of the opsin G protein-coupled receptor. In this work we measure the ligand-free dimerization of plexin receptors in live cell membranes and the response of the receptor to semaphorin-induced activation.

1300-Pos Board B251
Synthetic Manipulation of PIP2 Levels and PIP2-Associated Chemotactic Signaling Dissection in Dictyostelium
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Phosphatidylinositol 4,5-bisphosphate (PIP2) has been shown involved in key chemotactic signaling pathways in Dictyostelium, but our understanding of its roles in this signaling network is very limited. To explore the hypothesis that PIP2 negatively regulate chemotactic signaling events, we used the Chemically-induced Dimerization system, which allows inducible translocation of cellular proteins, to synthetically manipulate PIP2 level and dissect the signaling events. In my current data, after I synthetically recruited the PIP2-specific 5-phosphotase Inp54p to the plasma membrane, the PIP2 biosensor PHplcdelta fell off the membrane and the cells robustly oscillated between a spreading and a crunching morphology. Based on the localization changes of PIP2-binding protein PTEN, the PIP2 levels may further go down as cells spread, and rebound as cells crunch. In the same time, cells with the spreading morphology, but not the other one, have clearly activated chemotactic signaling events as shown by Ras and PIP3 biosensors, as well as highly elevated F-actin along the periphery. Further, cells still carried out this response when PIP3 production was diminished by the PI 3-Kinase inhibitor LY. Remarkably, these signaling events seemed to prevail when actin polymerization was greatly inhibited, as the signaling molecule PTEN showed a distinct spiral wave pattern in LatA-treated cells. These suggest PIP2 negatively regulates the chemotactic signaling network, at least partially independent of actin. We propose that PIP2 serves as an inhibitory role on the upstream chemotactic signaling molecule Ras; when PIP2 levels are decreased by Inp54p recruitment, multiple parallel chemotactic signaling pathways get activated as a result of Ras activation. Also in combine with previous evidence, we propose that PI 5-Kinase activation is downstream of PKBs activation, which up-regulates PIP2 levels, shuts down the signaling events and leads to the crunching morphology following spreading.

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Charge Shielding of PIP2 by Cations Regulates Enzyme Activity of Phospholipase C
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Hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) of the plasma membrane by phospholipase C (PLC) generates two critical second messengers, inositol-1,4,5-trisphosphate and diacylglycerol. For the enzymatic reaction, PIP2 binds to positively charged amino acids in the pleckstrin homology domain of PLC. Here we tested the hypothesis that positively charged polycations accumulate around the negatively charged PIP2, a process called electrostatic charge shielding, and therefore inhibit electrostatic PIP2-PLC interaction. Perfusion of the cations into cells via patch clamp pipette reduced PIP2 hydrolysis by PLC as triggered by M1 muscarinic receptors in the charge density-dependent manner. Accumulation of divalent cations into cells through divalent-permeable TRPM7 channel had the same effect. This charge shielding of PIP2 was measured quantitatively with an in vitro enzyme assay using WH-15, a water-soluble analog of PIP2, and various recombinant PLC proteins. Reduction of PLC activity by multivalent cations was well described by a theoretical model considering accumulation of cations around PIP2 via their electrostatic interaction and chemical binding. Altogether we suggest that endogenous divalent and multivalent cations modulate the activity of PLCs by controlling the amount of free PIP2 available for the enzymes. This work is supported by National Institutes of Health grant (DK080840).

1302-Pos Board B253
Soluble and Immobilized Vegf Induce Distinct Patterns of VEGFR2 Phosphorylation Mediated by Intracellular Trafficking
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Vascular endothelial growth factor (VEGF) is an important regulator of blood vessel growth. Matrix-binding and non-matrix-binding isoforms of VEGF stimulate production of blood vessel networks that are structurally and functionally different. Here, we develop a computational model of the binding of soluble and immobilized ligands to VEGF receptor 2 (VEGFR2), endosomal trafficking of VEGFR2, and site-specific VEGFR2 tyrosine phosphorylation to study differences in induced signaling between these VEGF isoforms. We build our model using experimental data in multiple cell lines, under different conditions, from several groups. In capturing essential features of VEGFR2 signaling and trafficking, our model suggests that VEGFR2 trafficking parameters are largely consistent across multiple endothelial cell lines. Simulations demonstrate distinct cellular localization of VEGFR2 phosphorylated on tyrosines 1175 and 1214. This is the first model to clearly show that differences in site-specific VEGFR2 activation when stimulated with immobilized VEGF compared to soluble VEGF can be accounted for by altered trafficking of VEGFR2 without an intrinsic difference in receptor activation. The model predicts that Neuropilin-1 can induce differences in the surface-to-internal distribution of VEGFR2, and that ligated VEGFR2 and phosphorylated VEGFR2 levels diverge over time following stimulation. We validated our model by successfully predicting changes in VEGFR2 phosphorylation resulting from perturbations of multiple phosphatases acting on VEGFR2. Using this model, we identify multiple key levers that alter how VEGF binding to VEGFR2 results in different coordinated patterns of multiple downstream signaling pathways. Specifically, simulations predict that perturbations of VEGFR2 trafficking (e.g. via VEGF immobilization), interactions with Neuropilin-1, and changes in expression or activity of phosphatases acting on VEGFR2 all affect the magnitude, duration, and relative strength of VEGFR2 phosphorylation on Y1175 and Y1214, and they do so predictably within our single consistent model framework.

1303-Pos Board B254
Measuring the Energetics of EphA3 Dimerization in Live Mammalian Cells
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EphA3 is a member of the largest family of receptor tyrosine kinases, the Eph receptor family. Mutations in EphA3 are known to cause lung, colorectal and hepatocellular cancers. Unlike other subfamilies of RTKs, the Eph family receptors form clusters upon binding to their ligands. However, the interactions of EphA3 in the absence of ligand are not well characterized. We used two-photon microscopy in conjunction with spectral FRET to characterize the dimerization of EphA3 in the absence of ligand in HEK293T cells. We measured the dimerization propensity of EphA3 and compared it to the dimerization of the EphA2 receptor. Our results show that unliganded EphA3 dimers are more stable than unliganded EphA2 dimers.

1304-Pos Board B255
Local Bilayer Reorganisation by the JM Regions of All Human RTKs: A Multiscale Molecular Dynamics Study
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The functional activity of membrane proteins is known to be modulated by lipids in the surrounding bilayer. In particular, the activity of the epidermal growth factor receptor 1 (EGFR) has recently been shown to be regulated by both GM3 in the outer leaflet[1], and by PIP_2 in the inner leaflet[2]. Additionally,